Total Synthesis of 7-*N*-Acetyldemethyllavendamycin *sec*-Butyl Ester and 7-*N*-Acetyldemethyllavendamycin *sec*-Butyl Amide

An Honors 499 Thesis

by

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May 8, 1997

Expected Date of Graduation: May 10, 1997

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I. Abstract

This thesis is part of ongoing research in the structure-activity relationship studies of various lavendamycin analogs as possible chemotherapeutic agents. The specific work contained in this thesis deals with two very similar analogs of lavendamycin: 7-*N*-acetydemethyllavendamycin *sec*-butyl ester and amide. By synthesizing these two similar analogs and eventually testing them for biological activity, a better understanding of the effects of structure on activity can be obtained. These structure-activity relationship studies are designed to lead to analogs of lavendamycin that have a high selective toxicity for ras^{k} oncogene transformed cells.

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II. Acknowledgments

I have a great appreciation for Dr. Mohammad Behforouz, a man who has been my teacher, mentor, and friend. I believe that the greatest learning experience I have had in college has not been in the classroom, but in the laboratory. This wonderful learning experience could not have been possible without the guidance of Dr. Behforouz; I am truly grateful for the many opportunities he has provided me with.

Wen Cai has helped my research on a daily basis since I began my in the spring of 1995. I want to thank her for her time and wisdom, because she was never too busy to help me with my problems. Her selfless attitude and extensive lab knowledge has helped this project tremendously. In addition, I would like to thank Mrs. Jayana Lineswala, a former graduate student in our research group. She has previously made several compounds similar to the ones discussed in this thesis, and I have used her M.S. thesis many times as a reference source.

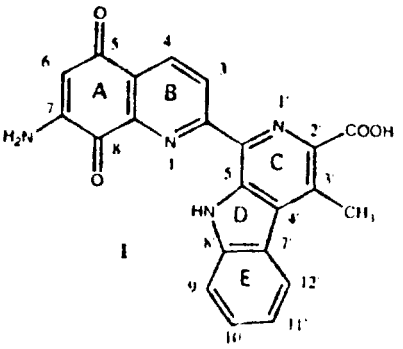
I would also like to thank the Ball State Chemistry Department for the vast amount of knowledge I have gained from them. It takes excellent professors to produce excellent students, and I believe that both exist here at Ball State. I commend all the faculty for the wonderful classes and research help I have had over the past four years.

I am also grateful for the funding of this work from many of our sources, including the National Institute of Health, the American Cancer Society, and Eli Lilly.

Finally, I would like to recognize Gary Rose, my father. His fortitude is comparable to the greatest of mountains; I have learned much from him.

III. Background Information

Lavendamycin (**1**) was first discovered in the fermentation broth of the microorganism *Streptomyces lavendulae* by researchers at Bristol Laboratories in 1981.[1] Lavendamycin was shown to be quite similar to the previously discovered compound, streptonigrin (**2**), in relation to their biological activities and structure. Both lavendamycin and streptonigrin have been shown to exhibit potent antitumor activity, but have been unusable thus far due to their high degree of cytotoxicity.[2, 3, 4] Lavendamycin also exhibits low water solubility, which makes it even less suitable for clinical applications.[1]



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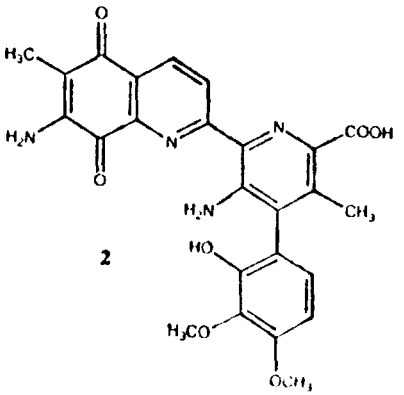

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Several mechanisms have been proposed for the cytotoxicity of streptonigrin and other quinones. DNA cleavage has been one observed result of quinones, which is believed to be correlated to the quinone reduction potential.[5] Other mechanisms have dealt with the effects of the compounds on the electron transport system within the mitochondria.[6-10] However, no single mechanism has fully elucidated the effects of these compounds as cytotoxic agents.

Streptonigrin has much greater cytotoxic and antitumor activity than many quinoline-5,8-diones that have been tested.[7] Preliminary studies by our research group have shown that

lavendamycins are more potent than their smaller quinolinedione analogs (the A-B ring portion of lavendamycin). While lavendamycin has been shown to be less potent than its counterpart streptonigrin, it has exhibited promising antitumor activities against $\underline{ras}^{K}$ oncogenic tumors.[11, 12]

Until recently, efficient methods of preparing lavendamycin analogs have been nonexistent. After the discovery of lavendamycin in 1981, several research groups started to work on methods to synthesize the entire pentacylic structure of lavendamycin. The first published synthesis of lavendamycin methyl ester originated from the work of Kende *et al* at the University of Rochester.[13, 14] Their method involved a condensation to acquire the A-B ring portion and Bischler-Napieralski cyclodehydration to produce the complete, pentacyclic product. Other methods evolved through the years, one of which involved a Pictet-Spengler condensation to add β-methyl tryptophan and a quinoline analog to form the main skeleton of the compound.[15]

In 1993, Behforouz's group at Ball State University reported a short and practical synthesis of lavendamycin methyl ester, using a novel azadiene Diels-Alder reaction followed by a condensation.[16] This synthesis consisted of five steps with an overall yield of 33%. Recently, Behforouz's group has introduced another five step synthesis to increase the overall yield to nearly 40%.[11, 17]

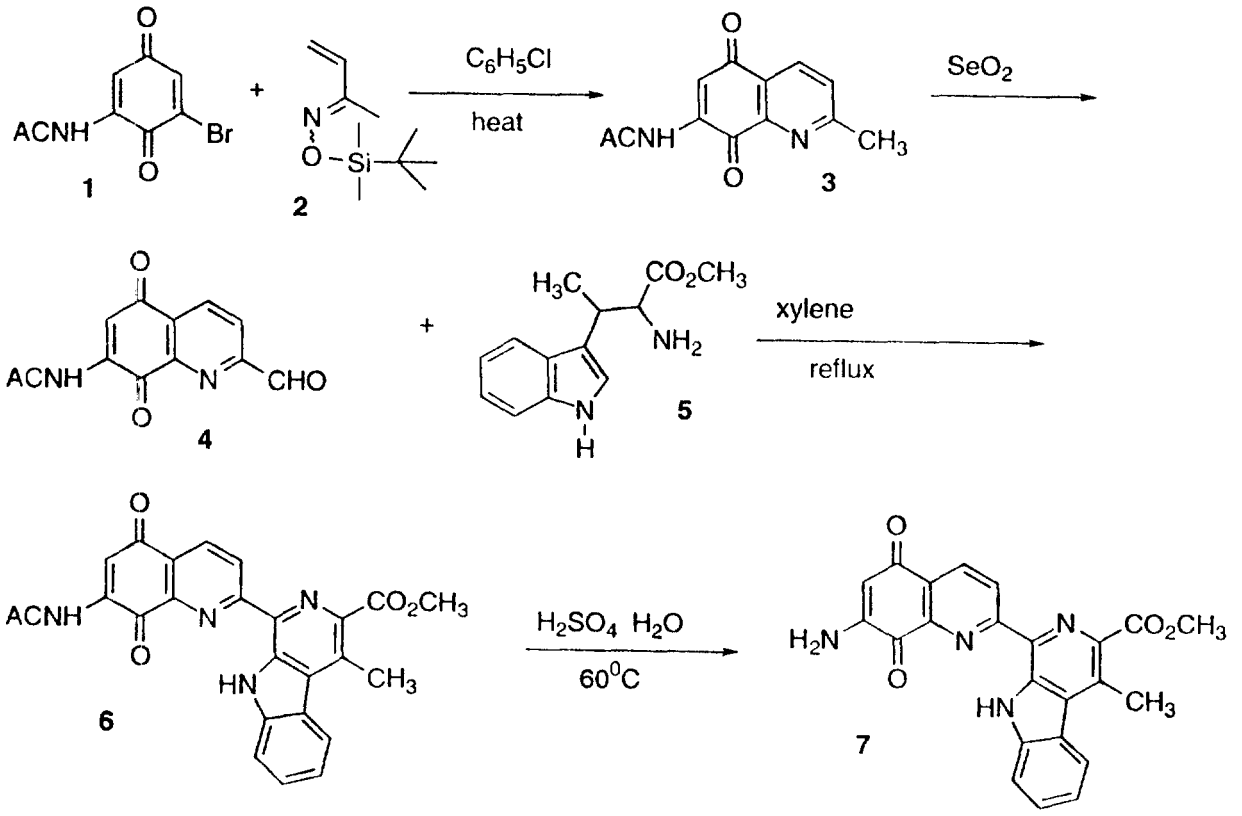
Behforouz's research group has been using this efficient synthesis for several years now, producing many analogs of the antitumor agent lavendamycin. Structure-activity relationship studies have been ongoing by Behforouz's group. These studies are focused upon finding the relationship between structure and the biological activity of the compounds.

IV. Synthesis of Lavendamycin Analogs

Since its discovery in 1981, lavendamycin has been synthesized by several research groups. In 1984, Kende and Ebetino at the University of Rochester reported the synthesis of lavendamycin methyl ester using a Friedlander condensation to produce the A-B ring portion and a Bischler-Napierski cyclodehydration to make the final pentacyclic product.[13] In addition, they reported using β-methyl tryptophan as an intermediate in their condensation. Next, Hibino's research group reported the synthesis of lavendamycin methyl ester in 1985.[15] They reported the use of a Pictet-Spengler condensation between β-methyl tryptophan and a quinoline analog to produce a pentacyclic intermediate, which was functionalized to the final product by a series of transformations. In 1985, Boger's group reported a total synthesis also, involving twenty steps with an overall yield of less than 1%.[18]

In 1993, Behforouz's group reported a highly concise synthesis of lavendamycin methyl

1993 Procedural Scheme



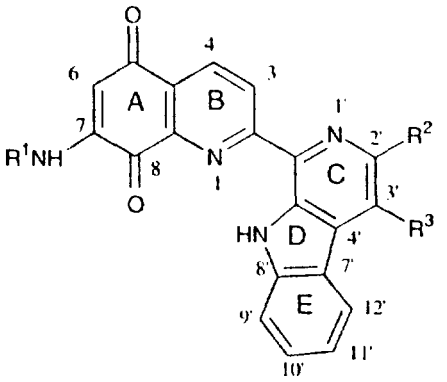
ester (**7**), which involved five steps and an overall yield of 33% (see 1993 Procedural Scheme).[16] In comparison to previously reported syntheses, Behforouz's method is much more practical in terms of number of intermediates, intermediate stabilities, and overall yield. This synthesis involved the use of a Diels-Alder condensation of bromoquinone **1** and a novel 1-azadiene **2** to make the AB ring portion **3**. After oxidizing the methyl group of the quinolinedione to an aldehyde **4**, a Pictet-Spengler condensation with β-methyl tryptophan (**5**) was performed to produce 7-*N*-acetyllavendamycin methyl ester (**6**), which was hydrolyzed to produce the final compound **7**.

In 1996, Behforouz's group reported an even more concise method of producing lavendamycin methyl ester.[17] This method has an overall yield of 40% and uses 8-hydroxyquinoline as a starting material for the synthesis of the A-B ring portion of lavendamycin (see Scheme 1). This improved method is, in fact, the one utilized in this thesis for the synthesis of two lavendamycin analogs.

V. Biological Activity

Structure-activity relationship studies on lavendamycin and a variety of analogs have been ongoing by Behforouz's group for several years. This has only been possible because of concise methods to produce lavendamycin and its analogs in good overall yields. Several analogs of lavendamycin have been found to be biologically active, including **7**, **8**, **9**, and **10** with 3, 9, 18, and 130 fold activity against $\underline{ras}^{K}$ oncogene transformed cells (see table below).[12] These biological results have been provided by the National Institute of Health, Eli Lilly, and several other

collaborators. So far, the SAR studies have shown that the acetamido group at the C-7 position is required for the most selective toxicity. In addition, amide and ester functional groups at the C-2' position have proven to contribute a great deal of biological activity to the compounds. Thus, the purpose of this thesis was to synthesize two analogs, both with the C-7 acetamido group and either an ester or an amide group at the C-2' position. By synthesizing these two



These four analogs have shown promising antitumor activity against oncogene-transformed cell lines

Analog	R^1	R^2	R^3	NRK	K/1	H/1.2	N/4.2	3LL
7	H	CO_2CH_3	CH_3	.1	.031	.11	.06	.25
8	CH_3CO	CO_2CH_3	CH_3	.9	.10	.70	N.T.	>33
9	CH_3CO	$CO_2(CH_2)_2CH(CH_3)_2$	H	1.62	.09	1.42	1.50	1.69
10	CH_3CO	$CO_2C_8H_{17}$ - *n*	H	>33	.25	7.60	9.00	>33

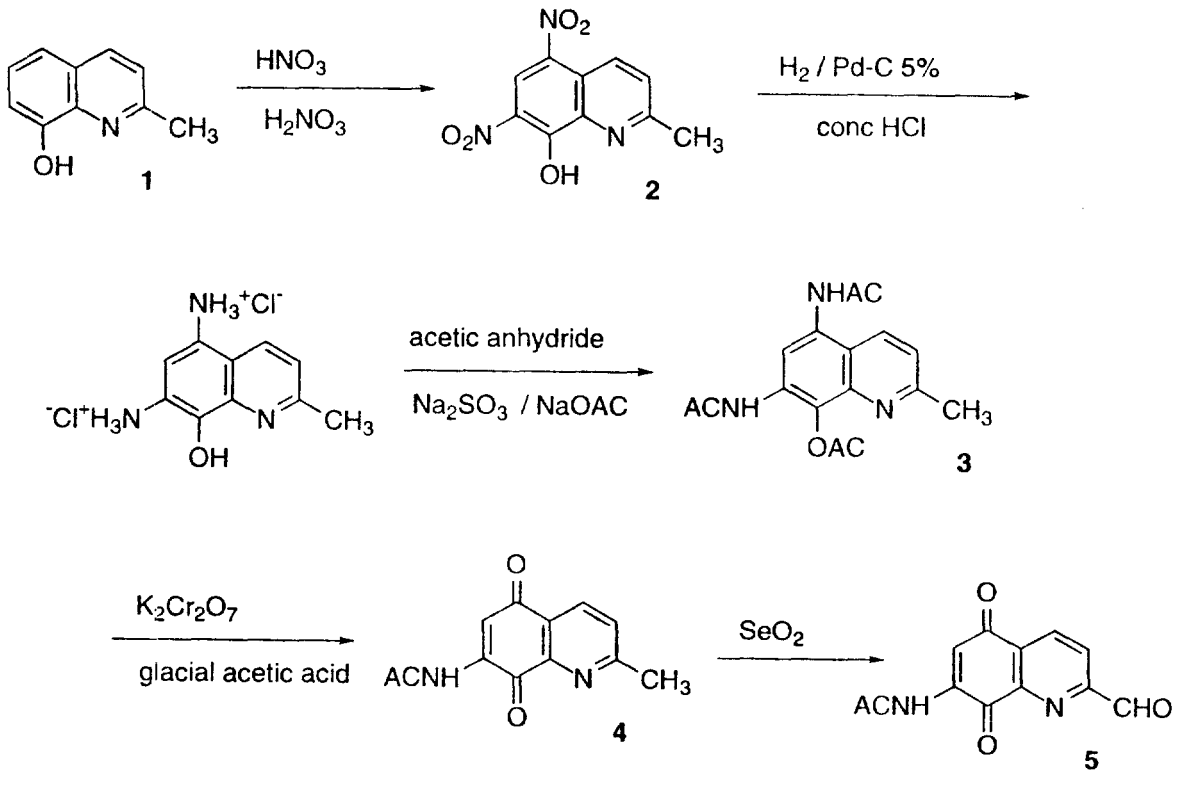
analogs and obtaining their antitumor activity data against various oncogene-transformed cell lines, we will be able to elucidate the roles that the ester and amide functional groups at the C-2' position play in relation to activity. Also, a comparison between these compounds and other ester/amide containing analogs should help provide additional data on the role of the residue group (such as *sec*-butyl) in relation to activity.

VI. Total Synthesis

The Pictet-Spengler condensation of a quinolinedione aldehyde and a tryptophan results in the final pentacyclic lavendamycin derivative. The quinoline aldehyde is fully functionalized prior to the condensation, as is the tryptophan. The solvent used for each condensation was carefully purified and dried prior to use, as were all reactants. The Pictet-Spengler condensation is believed to proceed through a spiroindolenine intermediate.[19]

The quinoline aldehyde was prepared in the following manner (refer to Scheme 1). 8-hydroxy-2-methylquinoline (**1**) was purchased from Aldrich, and a nitration yielded a dinitro product **2**. The nitration is performed with a concentrated nitric and sulfuric acid mixture. Since the hydroxyl group of 8-hydroxy-2-methylquinoline is an ortho-para director, the nitro groups are added accordingly. This reaction is quite exothermic, so an ice bath was used to keep the reaction temperature below 50^0 C.

Scheme 1



Next, the dinitro **2** was reduced to give the ammonium chloride salt. This reaction was performed using a Parr hydrogenator and 43 psi for 24 hours; HCl was placed in the hydrogenation mixture to change the reduced amino groups to ammonium chloride salts. This was necessary to help protect the resulting amino compound from oxidation (it is susceptible to air due to the electron contributing nature of the amino groups on the aromatic ring). The ammonium chloride salt was not isolated, but immediately placed into solution with sodium acetate, a base, and sodium sulfite, an antioxidant. The patient addition of acetic anhydride over time afforded the diacetamido product **3**. This reaction can be explained by the nucleophilic attack of the amino groups on the carbonyl carbons of acetic anhydride.

The diacetamido compound **3** was then oxidized with potassium dichromate, in a solution of glacial acetic acid, to yield the quinolinedione **4**. An extraction of the aqueous reaction mixture with dichloromethane was performed, and the organic solution was neutralized with 5% $NaHCO_3$. Rotary evaporation of the organic extracts afforded the yellow product.

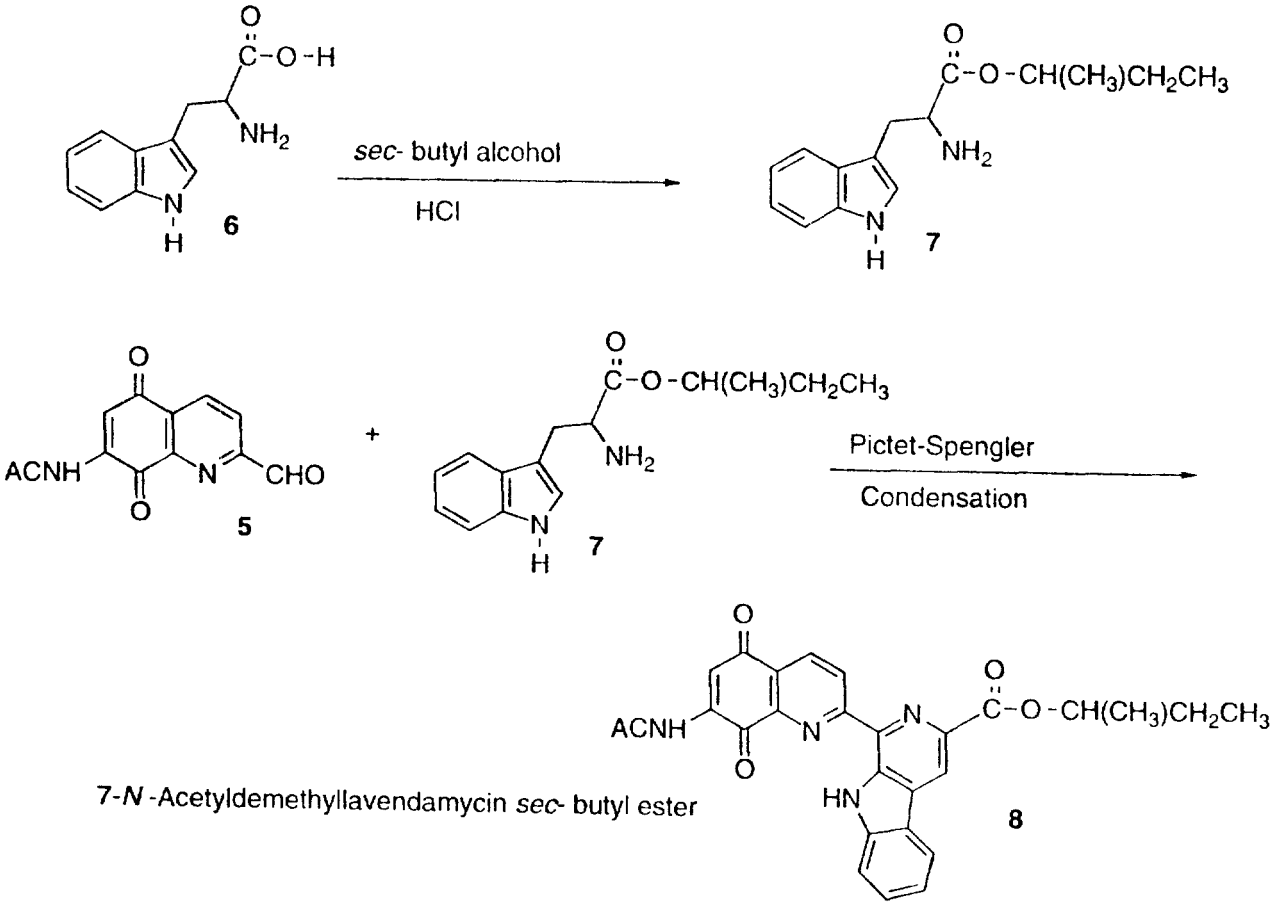
7-Acetamido-2-methylquinoline-5,8-dione (**4**) was then oxidized with selenium dioxide in a wet dioxane solution. This reaction required nearly 24 hours under an argon environment. The selenium dioxide and water react to form a reactive oxide of selenium, which serves to oxidize only the methyl group of the dione to an aldehyde. This quinoline aldehyde **5** was used to synthesize both analogs described in this thesis, and was produced with 17% yield overall.

The preparation of tryptophan *sec*-butyl ester (**7**) was performed in the following manner (refer to Scheme 2). (L)-Tryptophan (**6**), purchased from Aldrich, was refluxed with *sec*-butyl alcohol and a small amount of dry HCl for a period of 22 hours. This esterification afforded the

final tryptophan *sec*-butyl ester (**7**). Compound **7** was used in the Pictet-Spengler condensation, along with the quinoline aldehyde **5**, to produce 7-*N*-acetyldemethyllavendamycin *sec*-butyl ester (**8**) with approximately 7% yield overall.

The preparation of tryptophan *sec*-butyl amide (**12**) was performed in the following manner (see Scheme 3). *N*-Cbz tryptophan (**9**), purchased from Aldrich, was reacted with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide. This mixture was cooled and

Scheme 2

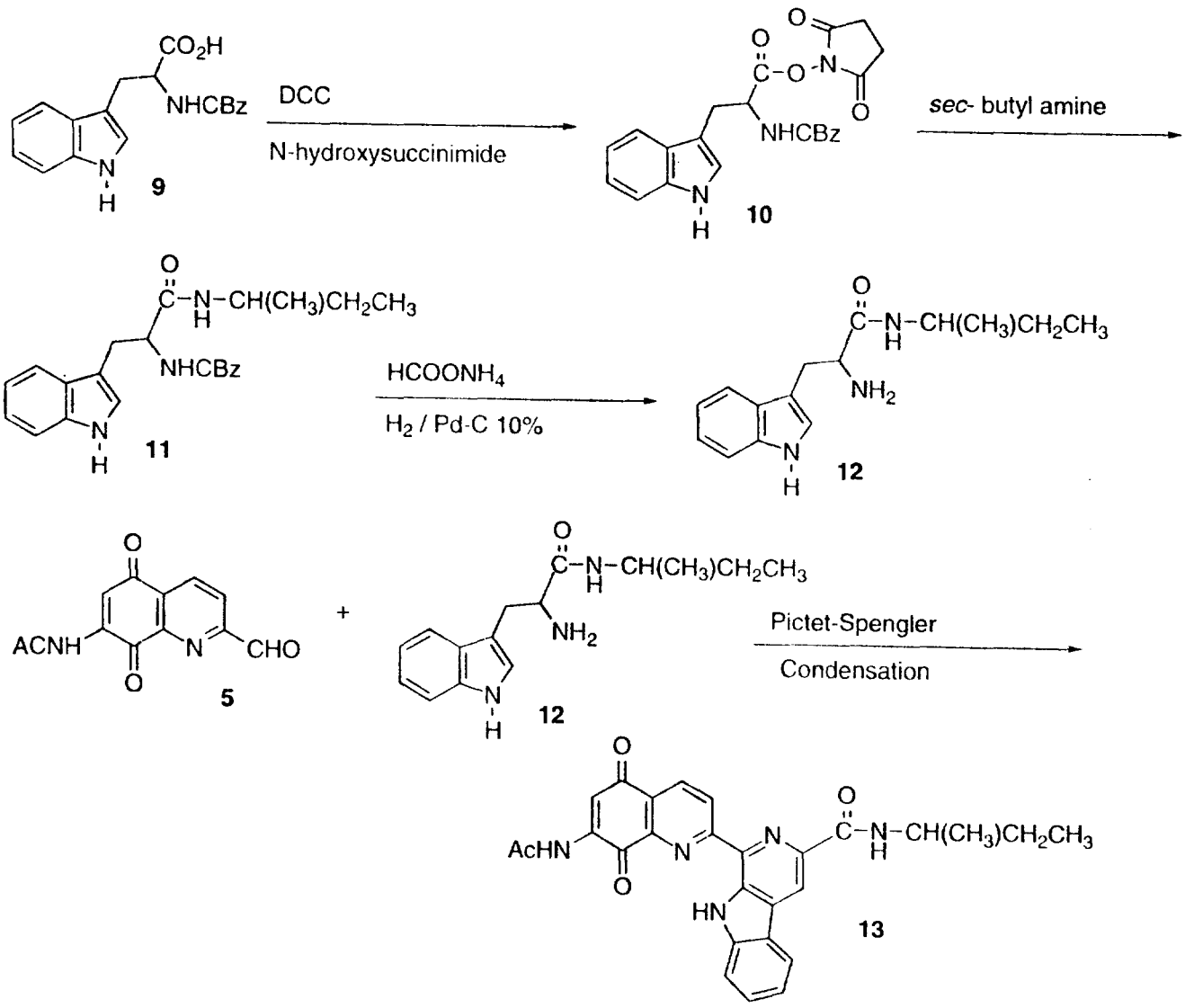


7-*N*-Acetyldemethyllavendamycin *sec*- butyl ester

stirred for two hours to afford the Cbz tryptophan succinimide ester (**10**). This compound was then mixed with a small amount of *sec*-butyl amine to replace the ester bond with an amide. This

reaction resulted in the formation of Cbz tryptophan *sec*-butyl amide (**11**). Next, the Cbz (carbobenzyloxy) protecting group was cleaved off using ammonium formate in the presence of 10% palladium on charcoal in methanol. This reaction resulted in the final tryptophan *sec*-butyl amide (**12**). Compound **12** was used in the Pictet-Spengler condensation, along with the quinoline aldehyde **5**, to produce 7-*N*-acetyldemethyllavendamycin *sec*-butyl amide (**13**).

Scheme 3



7-*N*-Acetyldemethyllavendamycin *sec*- butyl amide

VII. Experimental

A. General Information

Reagents: 8-hydroxy-2-methylquinoline, selenium dioxide, L-Tryptophan, *N*-carbobenzyloxytryptophan, *N*-hydroxysuccinimide, and *N*-dicyclohexylcarbodiimide were purchased from the Aldrich Chemical Company.

Solvents: All solvents used were reagent grade (except for 1,4-dioxane, xylene, and anisole, which were dried before use, see below)

Melting Points: All melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected.

NMR Spectra: ^{1}H NMR Spectra were recorded on a Varian Gemini 200 Spectrometer in $CDCl_3$ using TMS as an internal standard.

Low and High Resolution Mass Spectra: EI and FAB Mass Spectra were obtained at the Chemistry Department of the University of Illinois.

Thin-Layer Chromatography: Eastman silica gel strips with fluorescent indicator were used to determine purity for all products.

B. Solvent Purification

Xylene and anisole, the solvents used for the two condensation reactions, were carefully dried and purified. This involved refluxing the solvents with sodium spheres for 1-2 hours (or until the spheres appeared metallic), and then placing benzophenone in the reflux mixture. A blue color was indicative of dryness, and then the solvents were distilled.

It was quite necessary to purify and dry 1,4-dioxane prior to use because of its tendency to polymerize. To purify the solvent, it was refluxed with a large amount of potassium hydroxide. After decanting the solvent, it was dried by refluxing with sodium spheres and benzophenone as mentioned previously.

C. Procedures

Preparation of 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2)

Concentrated nitric (140 mL) and concentrated sulfuric (60 mL) acids were placed in a 500 mL Erlenmeyer flask and stirred while cooling with an ice bath. To this acid mixture, 8-hydroxyquinoline (**1**; 20.00 g, 0.125 mol) was carefully added in small portions over a period of 45 minutes while keeping the mixture between 20-30^{0}C. When the addition was complete, the reaction was left to stir at room temperature for 3 hours. Next, the reaction mixture was poured into a 2L beaker containing 800mL of ice water to produce a yellow precipitate instantly. The solid was filtered off, washed with water and diethyl ether to give 20.20g (64%) of product **2**. mp. 293-296^{0}C. ^{1}H NMR ($CDCl_3$): δ 9.65 (1H, d, J=9.1Hz, C-4**H**), 9.20 (1H, s, C-6**H**), 8.13 (1H, d, J=9.1Hz, C-3**H**), 2.93 (3H, s, C-2C**H**$_3$).

Preparation of 5,7-Diacetamido-2-methyl-8-acetoxyquinoline (3)

To a thick-walled 500 mL hydrogenation flask, finely ground 8-hydroxy-2-methyl-5,7-dinitroquinoline (**2**; 6.00 g, .024 mol) was added along with 13 mL of concentrated hydrochloric acid, 120 mL water, and 2.2 g 5% palladium-on-charcoal. The flask was quickly placed on a Parr hydrogenator and hydrogenated overnight, starting at 41 psi and falling to 30 psi. The next day, the reddish mixture was removed from the hydrogenator and filtered to remove the palladium-on-charcoal, which was rinsed with water (30 mL x 2). Moving quickly, 6 g of Na_2SO_3, 8 g of NaOAC, and a magnetic stir bar were added to the dark red filtrate; this solution was cooled and stirred vigorously while 90 mL of acetic anhydride was added dropwise. Once the addition was complete, the precipitate was filtered to give a whitish solid. Next, the filtrate was rotary-evaporated to 1/5 of its original volume, and another 25 mL of acetic anhydride was added. The resulting precipitate was filtered again, and the two precipitates were rinsed with water, combined and dried to give 5.87 g of product **3** (77%).

Preparation of 7-Acetamido-2-methylquinoline-5,8-dione (4)

Using a 500 mL Erlenmeyer flask containing a magnetic stir bar, 5,7-diacetamido-2-methyl-8-acetoxyquinoline (**3**) (2.73 g, .0087 mol) and 120 mL glacial acetic acid were mixed. Next, 8.8 g (.03 mol) of potassium dichromate was added and the mixture was stirred at room temperature for 24 hours. The reaction mixture was then added to a 2L separatory funnel containing 900 mL water, and extracted with dichloromethane (250 mL x 4). The combined organic extracts were concentrated by rotary-evaporation to nearly 200 mL, and then washed with 5% sodium bicarbonate (to pH 7). After drying over magnesium sulfate, the concentrated extracts were then rotary-evaporated to yield 2.01 g (51%) of yellow solid **4**. mp. 216-219^{0}C. ^{1}H NMR ($CDCl_3$): δ 8.41 (1H, s, C-N**H**), 8.32 (1H, d, J=8.1Hz, C-4**H**), 7.92 (1H, s, C-6**H**), 7.57 (1H, d, J=8.1Hz, C-3**H**), 2.78 (3H, s, C-2 CH_3), 2.33 (3H, s, NHCOCH_3).

Preparation of 7-Acetamido-2-formylquinoline-5,8-dione (5)

To a 100 mL round-bottomed flask equipped with a magnetic bar and argon flow, 7-acetamido-2-methylquinoline-5,8-dione (**4**; 1.15 g, 4.7 mmol), 1.08 g selenium dioxide, 17.5 mL of purified and dried dioxane, and 0.625 mL water were added. The mixture was slowly heated in an oil bath until constant reflux was achieved, and then refluxed for 20 hours. After determining that the reaction was completed using TLC (50/50 $CHCl_3$: EtOAC), an additional 15 mL of dioxane was added and refluxing was maintained for another 15 minutes. Next, the mixture was vacuum filtered while hot, and the selenium metal precipitate was washed with 25 mL of dichloromethane. The filtrate (containing product) was saved, and the selenium metal precipitate was placed in a beaker and thoroughly mixed with 50 mL dichloromethane while heating to a boil. This mixture was once again filtered, and the filtrates were combined and rotary-evaporated to yield a brown precipitate. After two days of vacuum drying, the solid was found to be 0.84 g (69%). mp 220-223^{0}C. ^{1}H NMR ($CDCl_3$): δ 10.31 (1H, s, ArC**H**O), 8.64 (1H, d, J=7.7Hz, C-4**H**), 8.07 (1H, s, C-6**H**), 8.34 (1H, d, J=7.7Hz, C-3**H**), 8.46 (1H, s, C-7N**H**), 2.37 (3H, s, $COCH_3$).

Preparation of Tryptophan *sec*-butyl ester (7)

L-Tryptophan (**6**; 1 g, 4.9 mmol) and 60 mL of *sec*-butyl alcohol were placed in a 100 mL round-bottomed flask equipped with a magnetic stirring bar, water condenser and an oil bubbler. The flask was cooled in an ice bath for several minutes, and then 12 mL of dry HCl-ether solution was added into the flask using a syringe through a rubber septum. The ice bath was then removed and replaced by an oil bath, and the mixture was heated to 120^{0}C and left to reflux for 22 hours while stirring vigorously. Next, 3 drops of concentrated sulfuric acid was added, and the mixture was refluxed for an additional two hours. TLC was used to determine the completion (100% EtOAC) of the reaction and the mixture was cooled and rotary-evaporated to dryness. The resulting solid was dissolved in 50 mL of dichloromethane, and then washed with 5% sodium bicarbonate until pH~ 8. This solution was extracted with dichloromethane (40 mL x 4), washed with brine, and dried over magnesium sulfate. Rotary-evaporation gave 0.88 g (69%) of a light brown gel (**7**). ^{1}H NMR ($CDCl_3$): δ 8.22 (1H, br s, -N**H**), 7.65 (1H, d, J=7.7Hz, C-4**H**), 7.25-7.1 (2H, m, C-5 and C-6**H**), 7.35 (1H, d, J=7.7Hz, C-7**H**), 7.05 (1H, s, C-2**H**), 3.87-3.75 (1H, m, RCOOC**H**R_2), 1.3 (2H, d, J=6.9Hz, RC$\mathbf{H}_2$R), .85 (3H, t, J=7.1Hz, $CH_2C\mathbf{H}_3$), 1.22-1.15 (2H, m, RCHC$\mathbf{H}_2CH_3$), .90 (3H, d, J=7.3Hz, CHC$\mathbf{H}_3$).

Preparation of 7-N-Acetyldemethyllavendamycin *sec*-butyl ester (8)

L-Tryptophan *sec*-butyl ester (**7**; 0.2568 g, 1.0 mmol) was dissolved in 12 mL of freshly distilled xylene and stored under argon in a closed container. To a 250 mL round-bottomed flask equipped for argon flow and a magnetic stirring bar, 7-acetamido-2-formylquinoline-5,8-dione (**5**; 0.2401 g) and 145 mL of freshly distilled xylene were placed and heated to 100^{0}C. The 15 mL tryptophan/xylene solution was then added to the flask using a syringe. This mixture was then heated to 130^{0}C and left to stir for 16 hours. After using TLC to determine the reaction's completion (CH_2Cl_2 : MeOH, 2:1), the mixture was filtered to reveal a green precipitate. The filtrate was left in the refrigerator overnight and the next day more precipitate was obtained by filtration. These precipitates were combined to give 0.2785 g (57%) of product. The precipitate

was recrystallized in dimethyl sulfoxide to yield the pure product **8**. mp 268^{0}C. ^{1}H NMR ($CDCl_3$): δ 11.86 (1H, br s, -N**H**), 9.27 (1H, d, J=8.4Hz, C-4**H**), 8.97 (1H, s, C-6**H**), 8.61 (1H, d, J=8.3Hz, C-3**H**), 8.47 (1H, br s, C-7N**H**), 8.29 (1H, d, J=8.1Hz, C-9'**H**), 8.03 (1H, s, C-3'**H**), 7.77-7.71 (2H, m, C-10'**H** and C-11'**H**), 7.40 (1H, d, J=9.7Hz, C-12'**H**), 5.35-5.20 (1H, m, COOC**H**R_2), 2.38 (3H, s, C**H**$_3$CONH), 1.50-1.44 (2H, m, RC**H**$_2$CH$_3$), 1.26 (3H, s, R_2CHC**H**$_3$), 1.10 (3H, t, J=7.3Hz, RCH$_2$C**H**$_3$). FAB-MS, m/z (relative intensity), 485.2 (24.75), 309.0 (17.73), 155.1 (57.69), 119.0 (100); HRMS m/e for $C_{27}H_{25}N_4O_5$. calculated 485.182495, found 485.182300.

Preparation of Benzyloxycarbonyltryptophan succinimide ester (10)

This procedure is similar to the method used by Tolstikov.[20]

To a 100 mL round-bottomed flask with a magnetic stirring bar, *N*-carbobenzyloxytryptophan (1.706 g, 5 mmol), 0.598 g (5.2 mmol) *N*-hydroxysuccinimide, and 50 mL of purified, dry dioxane were added. This mixture was stirred until it became homogenous, and then cooled to nearly 12^{0}C in an ice bath. Dicyclohexylcarbodiimide (1.04 g, 5 mmol) was added, and the ice bath was changed to a cold water bath. The reaction mixture was allowed to stir for 2 hours while maintaining a temperature of 15-20^{0}C. The water bath was then removed and the mixture was stirred for an additional two hours. After the completion of the reaction (TLC 2.5 mL EtOAc : 1.5mL hexane), the precipitate was filtered and the white solid was washed with a small amount of dioxane. The filtrate was rotary-evaporated to obtain a clear gel. The gel was placed under vacuum with heat (40^{0}C) for several days to obtain 1.81 g (82%) of a white solid product (**10**). ^{1}H NMR ($CDCl_3$): δ 8.25 (1H, br s, N-**H**), 7.55 (1H, d, J=5.76Hz, C--4**H**), 7.36 (1H, d, J=6.80Hz, C-7**H**), 7.27 (2H, d, C-5 and C-6**H**), 7.07 (1H, s, C-2**H**), 5.30 (1H, br s, CON**H**), 5.12 (2H, s, benzyl-C**H**$_2$-R), 3.76 (1H, m, NH-C**H**-R), 3.50-3.43 (2H, m, C-4' C**H**$_2$).

Preparation of Benzlyoxycarbonyltryptophan *sec*-butyl amide (11)

This procedure is similar to the method used by Tolstikov.[20]

To a 100 mL round-bottomed flask equipped for an argon flow and a magnetic stirring bar, benzyloxycarbonyltryptophan succinimide ester (**10**; 0.870 g, 2 mmol), 0.22 mL (2 mmol) of *sec*-butyl amine, 0.28 mL of purified triethylamine, 26 mL of absolute ethanol, and 24 mL of chloroform were placed. The reaction mixture was stirred at room temperature for two hours while monitoring the reaction's progress with TLC (1 mL EtOAc : 2 mL $CHCl_3$). Once completed, the mixture was rotary-evaporated to a clear gel. This gel was dissolved in 60 mL EtOAc and washed with 30 mL of water. Next, the solution was washed with 10% citric acid (25 mL x 2) and then with 12 mL of 1.2M $NaHCO_3$. The organic solution was then dried over sodium sulfate and rotary-evaporated to obtain a clear, brown gel. After two days under vacuum, 0.615 g (78%) of a white product **11** was obtained. ^{1}H NMR ($CDCl_3$): δ 8.1 (1H, br s, N-**H**), 7.71 (1H, d, J=5.86Hz, C--4**H**), 7.30 (1H, d, J=6.56Hz, C-7**H**), 7.27 (2H, dd, C-5 and C-6**H**), 7.03 (1H, s, C-2**H**), 5.5 (1H, br s, CON**H**), 5.30 (1H, d, CON**H**), 5.12 (2H, s, benzyl-C**H**$_2$-R), 4.43 (1H, t, J=5.5Hz, C-5'**H**), 3.76 (1H, m, NH-C**H**-R), 3.40-3.13 (2H, m, C-4' C**H**$_2$), 1.24 (2H, t, J=7.98Hz, -CH(CH_3)C**H**$_2$$CH_3$), .9 (3H, d, J=6.42, -CH(C**H**$_3$)CH_2CH_3), .79 (3H, t, J=6.60, CH(CH_3)CH_2C**H**$_3$).

Preparation of Tryptophan *sec*-butyl amide (12)

This procedure is similar to the method used by Tolstikov.[20]

A magnetic stirring bar and 0.5 g (1.3 mmol) of benzyloxycarbonyltryptophan *sec*-butyl amide (**11**) were placed in a 100 mL round-bottomed flask equipped for argon flow, along with 30 mL of dried methanol. To this mixture, 0.26 g (5.5 mmol) of dried ammonium formate and 0.26 g of 10% palladium on charcoal was added and the mixture was left to stir for 30 minutes under argon while monitoring with TLC (1 EtOAc : 2 $CHCl_3$). Once completed, the reaction mixture was filtered and the filter cake was washed with 10 mL of methanol. The filtrate was then rotary-evaporated until nearly dry, and then the water bath temperature was raised to 100°C to obtain a brownish precipitate. This precipitate was dried under vacuum in a 60°C oil bath for two days to obtain 0.26 g (75%) of white solid product **12**. ^{1}H NMR ($CDCl_3$): δ 8.32 (1H, br s, N-

H), 7.71 (1H, d, J=7.22Hz, C-4**H**), 7.12 (1H, d, J=8.44Hz, C-7**H**), 7.27 (2H, dd, C-5 and C-6**H**), 7.01 (1H, s, C-2**H**), 3.90 (1H, t, J=6.5Hz, C-5'**H**), 3.72-3.68 (1H, m ,C**H**$(CH_3)CH_2CH_3$), 3.45-2.90 (2H, m, C-4' C$\mathbf{H}_2$), 1.39 (2H, m, -CH(CH_3)C$\mathbf{H}_2CH_3$), 1.09 (3H, d, J=6.5, -CH(C$\mathbf{H}_3$)CH_2CH_3), .90 (3H, t, J=6.75, -CH$(CH_3)CH_2$C$\mathbf{H}_3$).

Preparation of 7-N-Acetyldemethyllavendamycin *sec*-butyl amide (13)

For this procedure a 250 mL round-bottomed flask, flowing argon, a magnetic stirring bar, and a Dean-Stark collector were used. 7-acetamido-2-formylquinoline-5,8-dione (**5**; 109 mg, 0.45 mmol) and tryptophan *sec*-butyl amide (**12**; 116.4 mg, 0.45 mmol) were placed in the flask, along with 180 mL of dried anisole. This mixture was stirred and heated, using an oil bath, to 167^{0}C over a period of two hours. The solution was refluxed for a total of 15 hours, and the completion of the reaction was verified by TLC (5% MeOH in $CHCl_3$). The mixture was filtered while hot to remove some brown solid impurity. The filtrate was rotary evaporated to dryness, and the resulting solid was dissolved in a minimal amount of $CHCl_3$ and acetone. This solution was set aside overnight at room temperature, and the following day filtration yielded 73 mg (35%) of orange product **13**. mp 306-308^{0}C. ^{1}H NMR ($CDCl_3$): δ 11.80 (1H, br s, N**H**), 9.10 (1H, s, C-3'**H**), 9.00 (1H, d, J=8.4Hz, C-4**H**), 8.63 (1H, d, J=8.4Hz, C-3**H**), 8.49 (1H, br s, C-7N**H**), 8.28 (1H, d, J=7.7Hz, C-11'**H**), 8.04 (1H, s, C-6**H**), 7.95-7.89 (1H, m, CON**H**-*sec*-butyl), 7.77-7.69 (2H, m, C-10'**H** and C-11'**H**), 7.41 (1H, d, J=8.3Hz, C-12'**H**), 2.39 (3H, s, NHCOC$\mathbf{H}_3$), 4.25-4.18 (1H, m, NHC**H**$(CH_3)CH_2CH_3$), 1.75-1.73 (2H, m, NHCH(CH_3)C$\mathbf{H}_2CH_3$), 1.39 (3H, d, J=6.56, NHCH(C$\mathbf{H}_3$)CH_2CH_3), 1.07 (3H, t, J=7.44Hz, NHCH$(CH_3)CH_2$C$\mathbf{H}_3$).

Appendix A

Index of Spectra

8-Hydroxy-2-methyl-5,7-dinitro quinoline.....(^{1}H NMR).....**(2)**

7-Acetamido-2-methylquinoline-5,8-dione.....(^{1}H NMR).....**(4)**

7-Acetamido-2-formylquinoline-5,8-dione.....(^{1}H NMR)..... **(5)**

Tryptophan *sec*-butyl ester.....(^{1}H NMR).....**(7)**

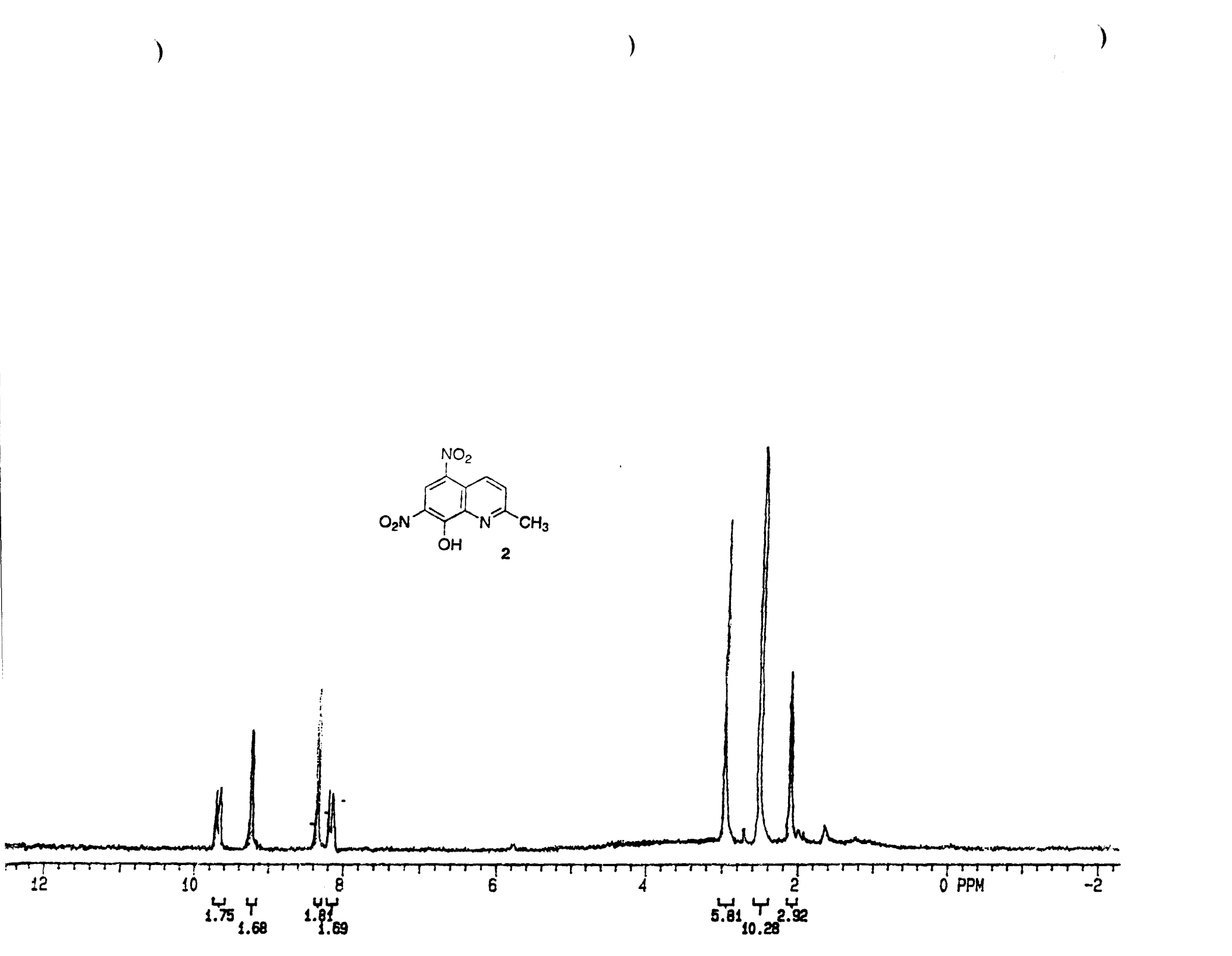
7-*N*-Acetyldemethyllavendamycin *sec*-butyl ester.....(^{1}H NMR).....**(8)**
(Mass Spec).....**(8)**

Benzyloxycarbonyltryptophan succinimide ester.....(^{1}H NMR).....**(10)**

Benzyloxycarbonyltryptophan *sec*-butyl amide.....(^{1}H NMR).....**(11)**

Tryptophan *sec*-butyl amide.....(^{1}H NMR).....**(12)**

7-*N*-Acetyldemethyllavendamycin *sec*-butyl amide...(^{1}H NMR).....**(13)**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10.28
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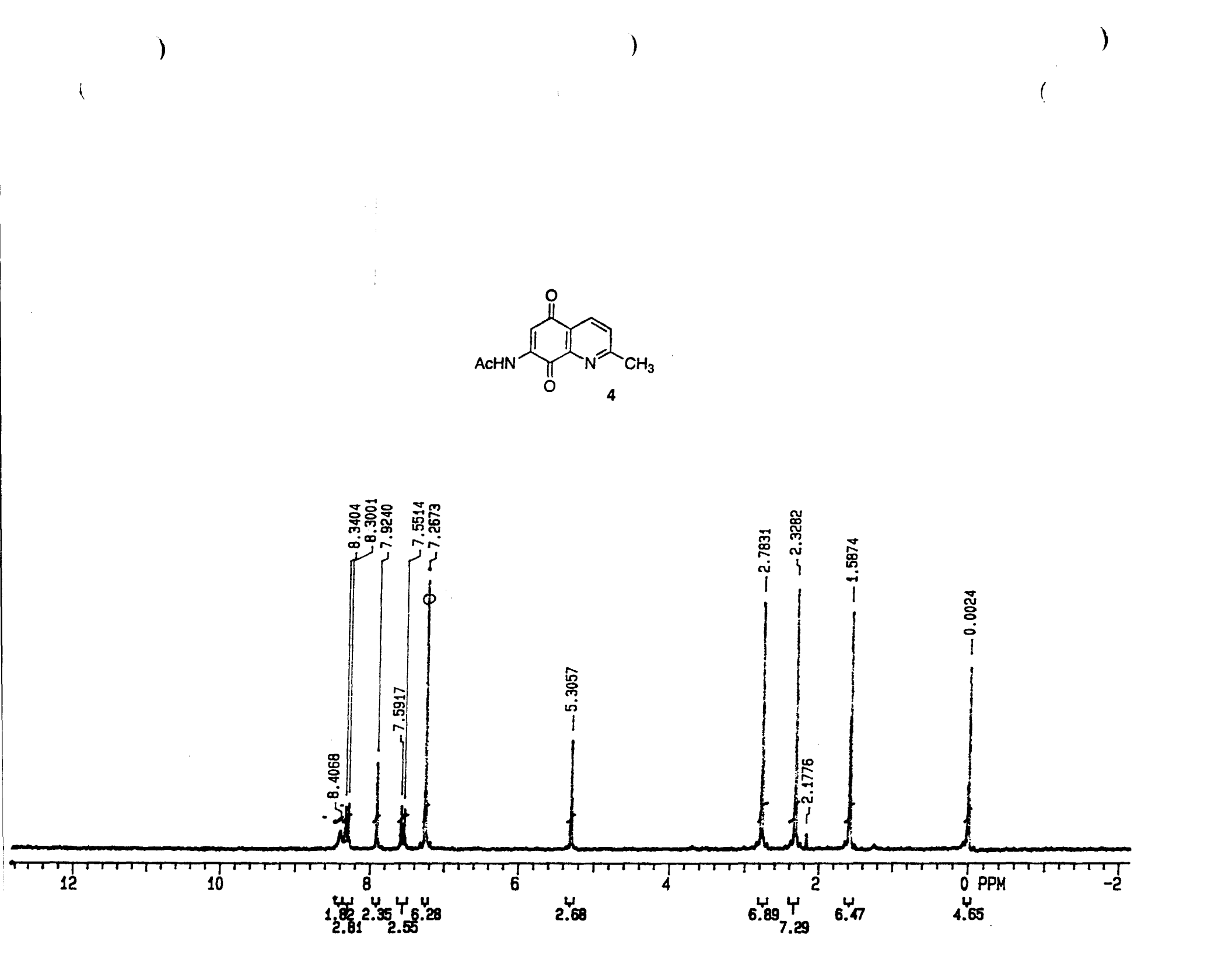
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0.0024
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10
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4
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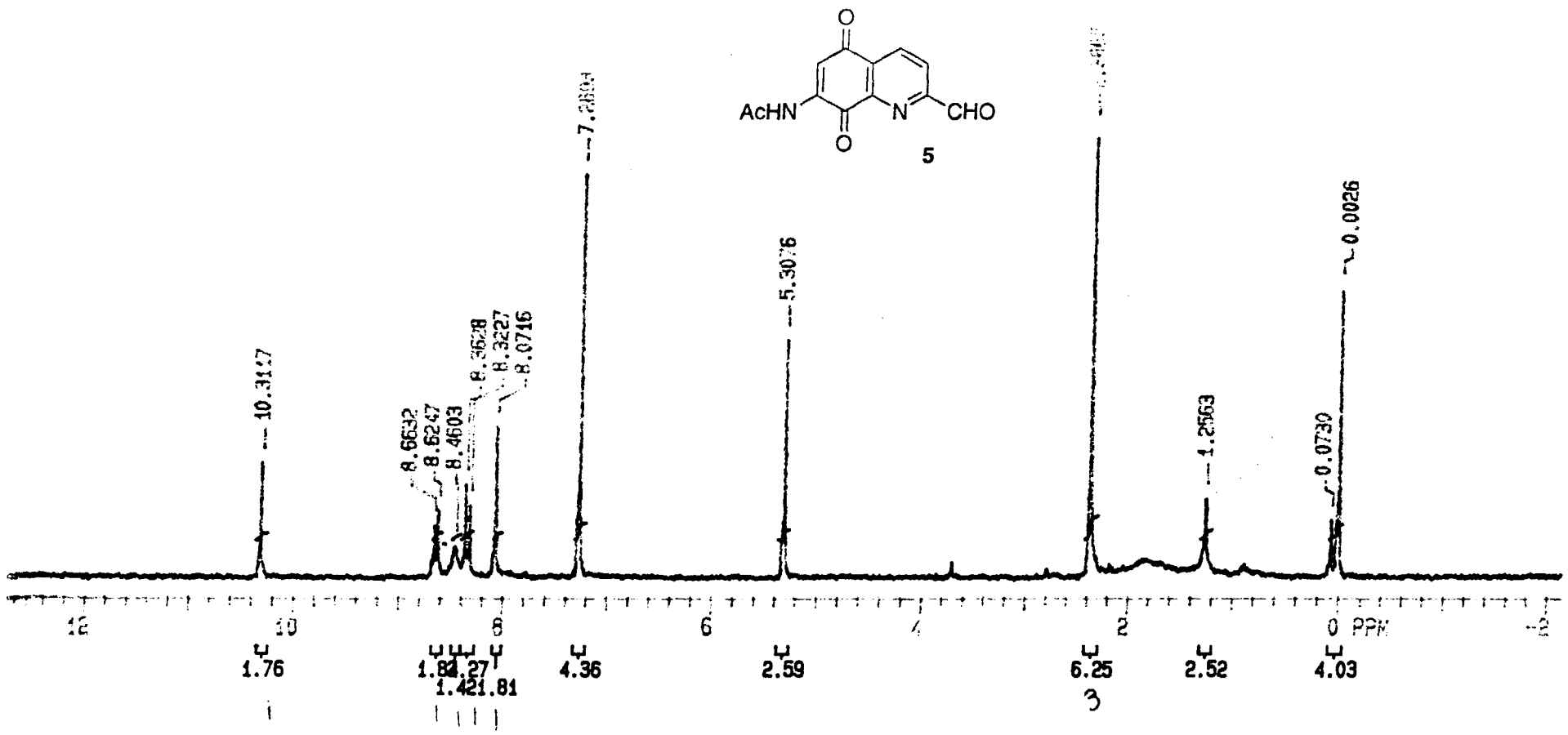
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6
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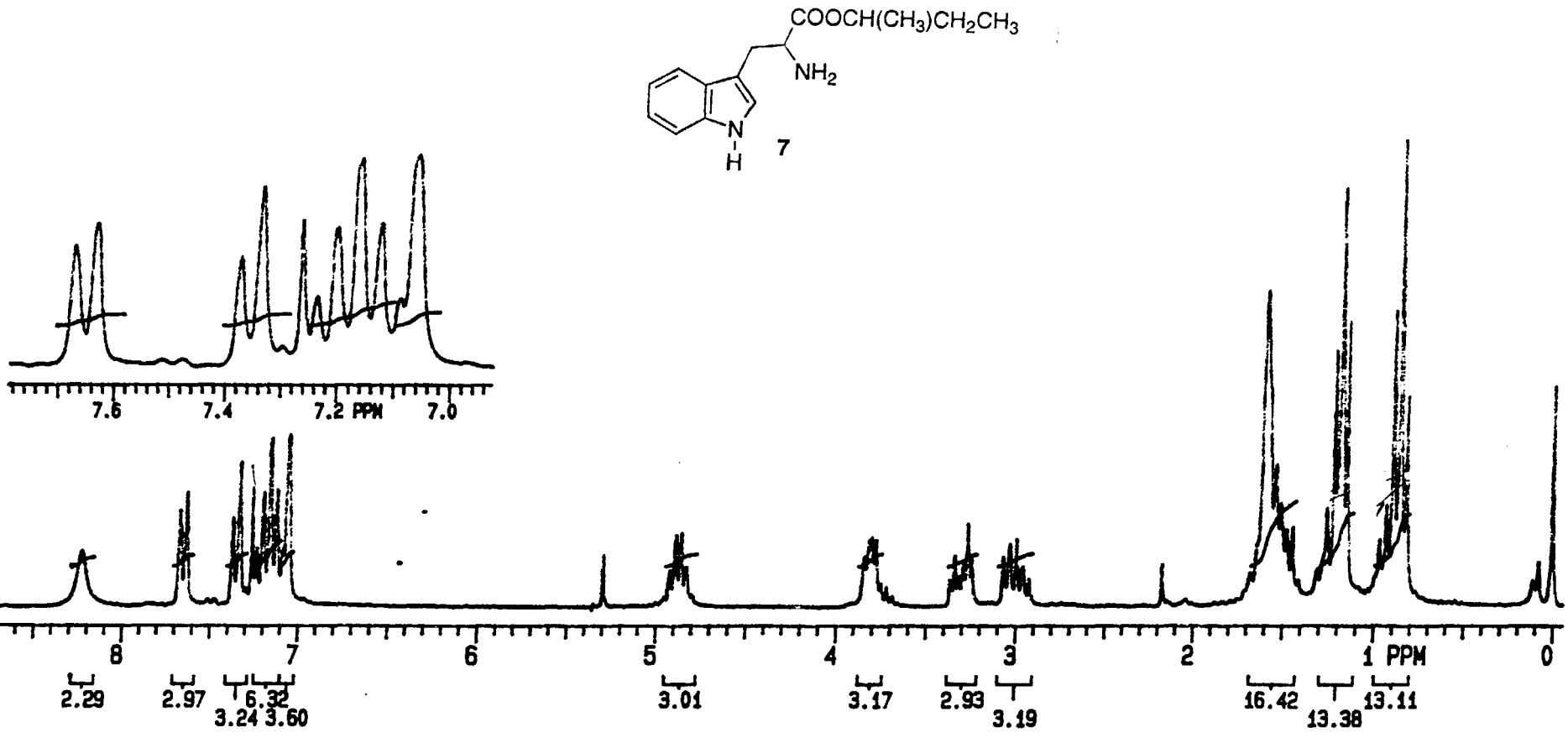
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7.2 PPM
7.0
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6
5
4
3
2
1 PPM
0
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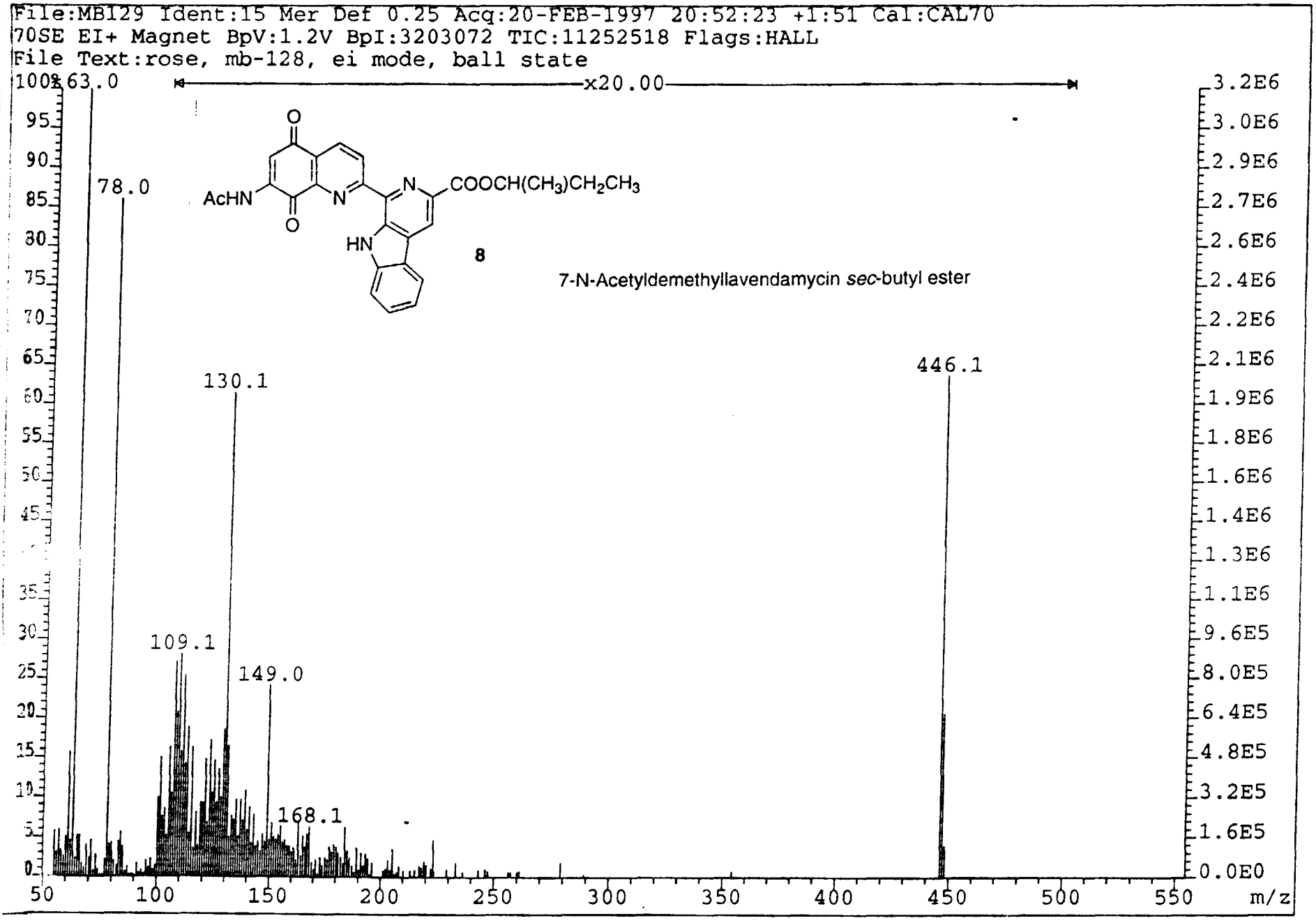
13.38
13.11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130.1
446.1
109.1
149.0
168.1
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COOCH(CH3)CH2CH3
HN
8
7-N-Acetyldemethyllavendamycin sec-butyl ester
m/z

Elemental Composition Date : 25-FEB-1997

Heteroatom Max: 40 Ion: Both Even and Odd
Limits:

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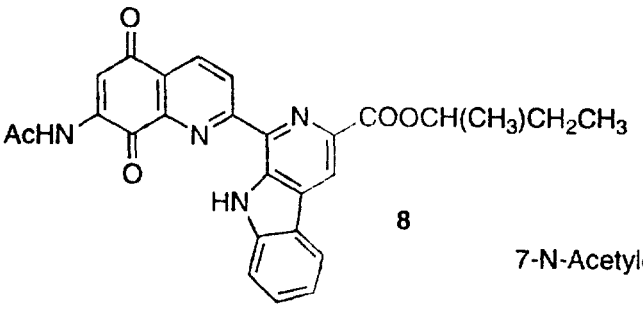

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7-N-Acetyldemethyllavendamycin *sec*-butyl ester

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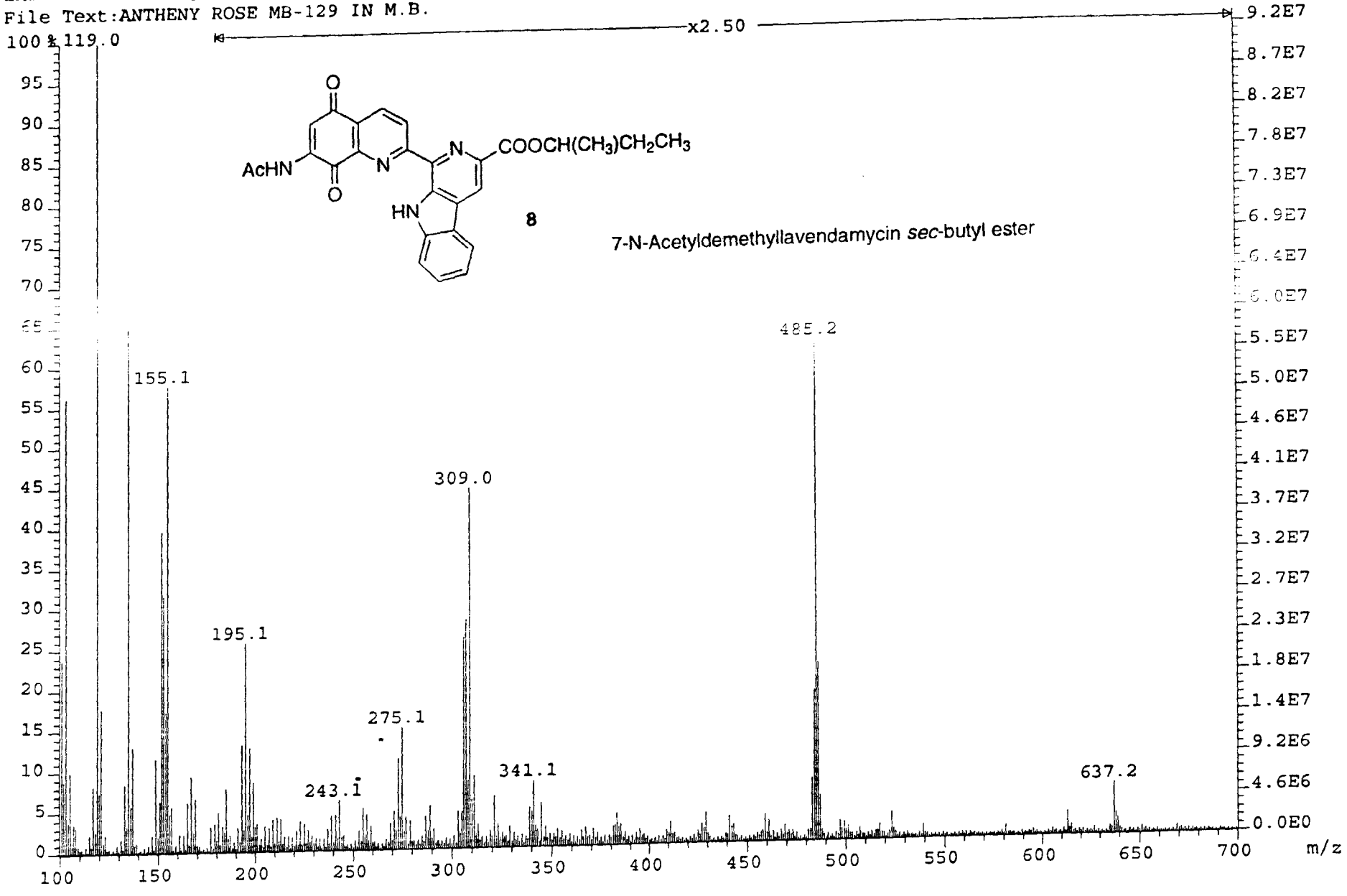

8

7-N-Acetyldemethyllavendamycin *sec*-butyl ester

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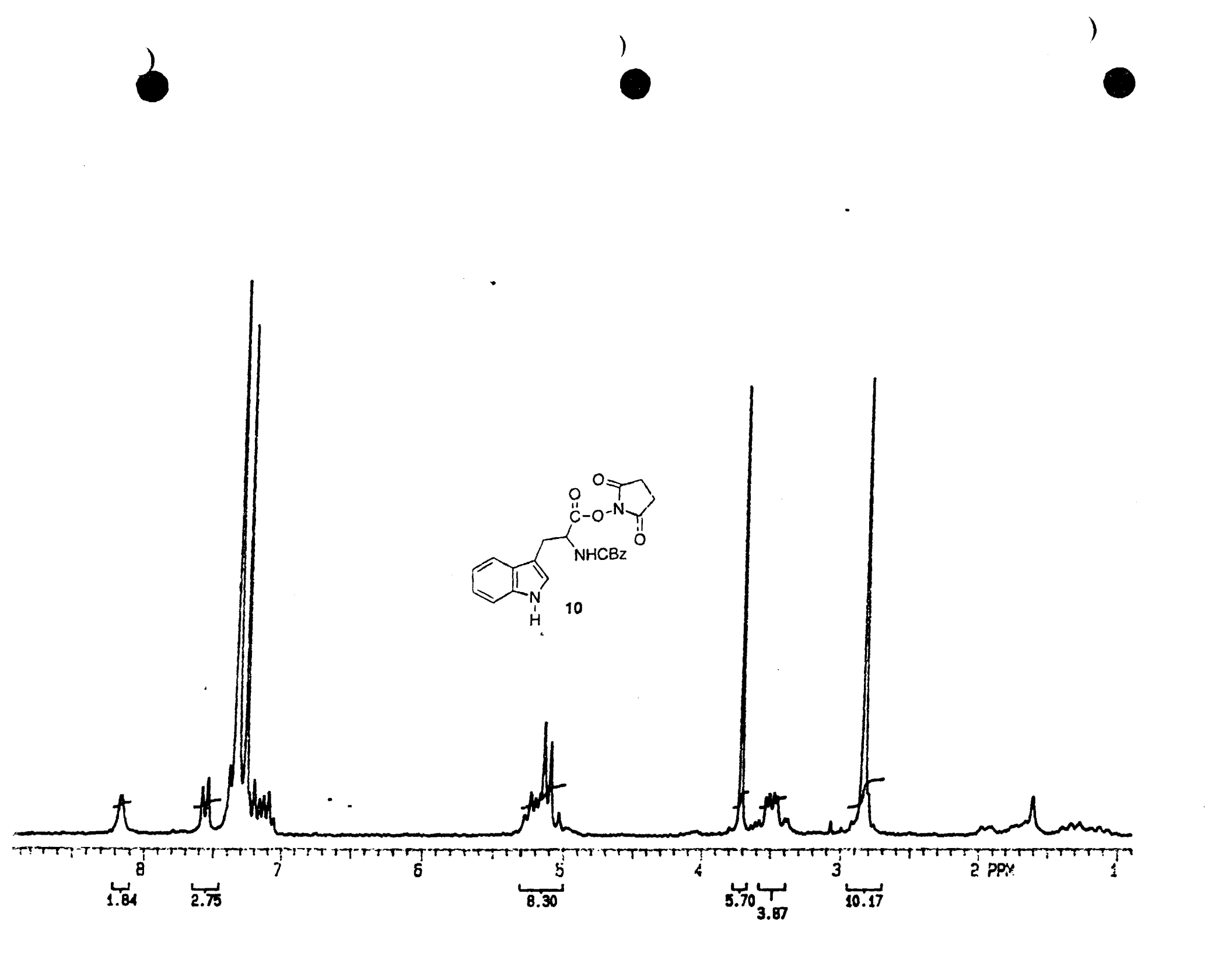
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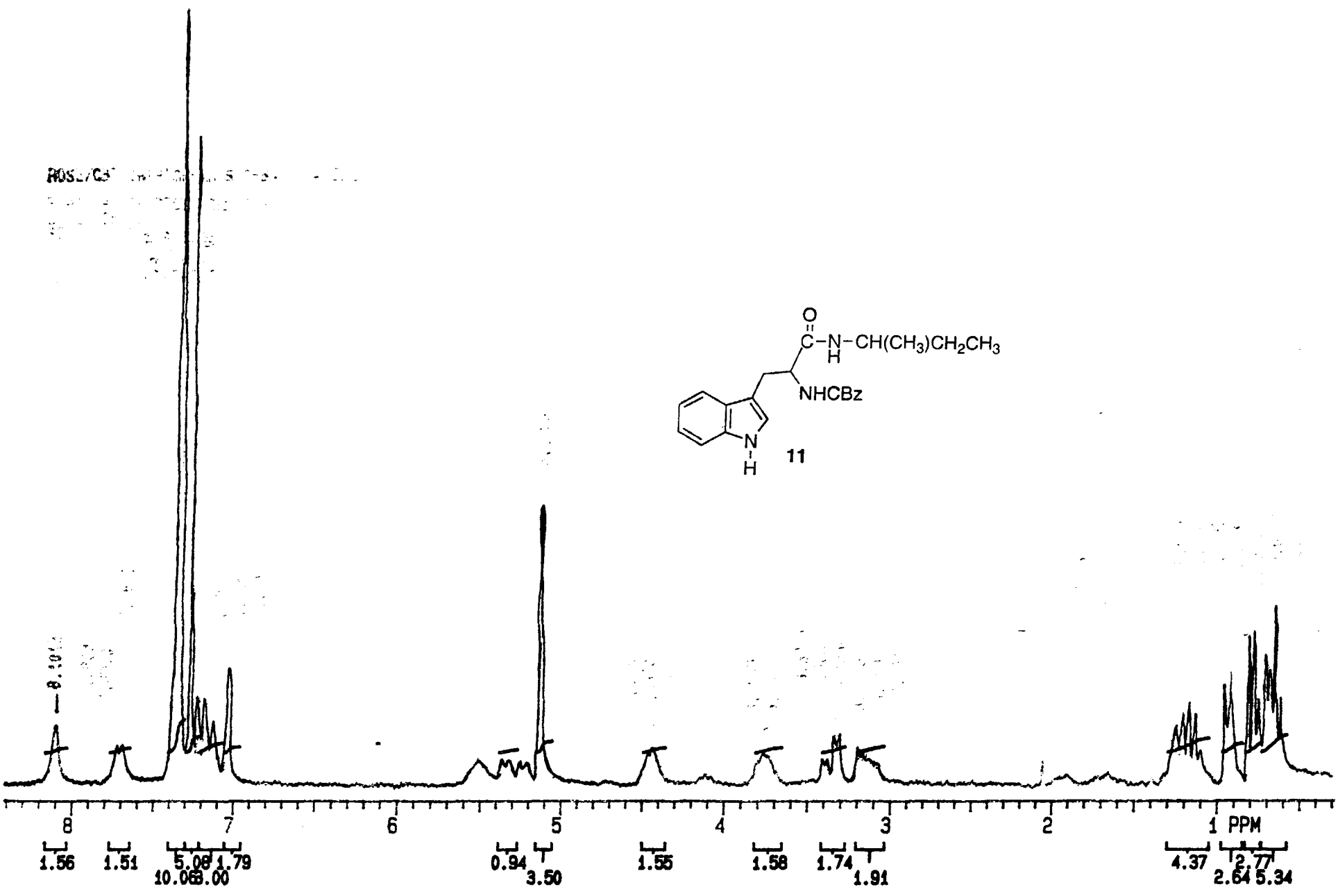
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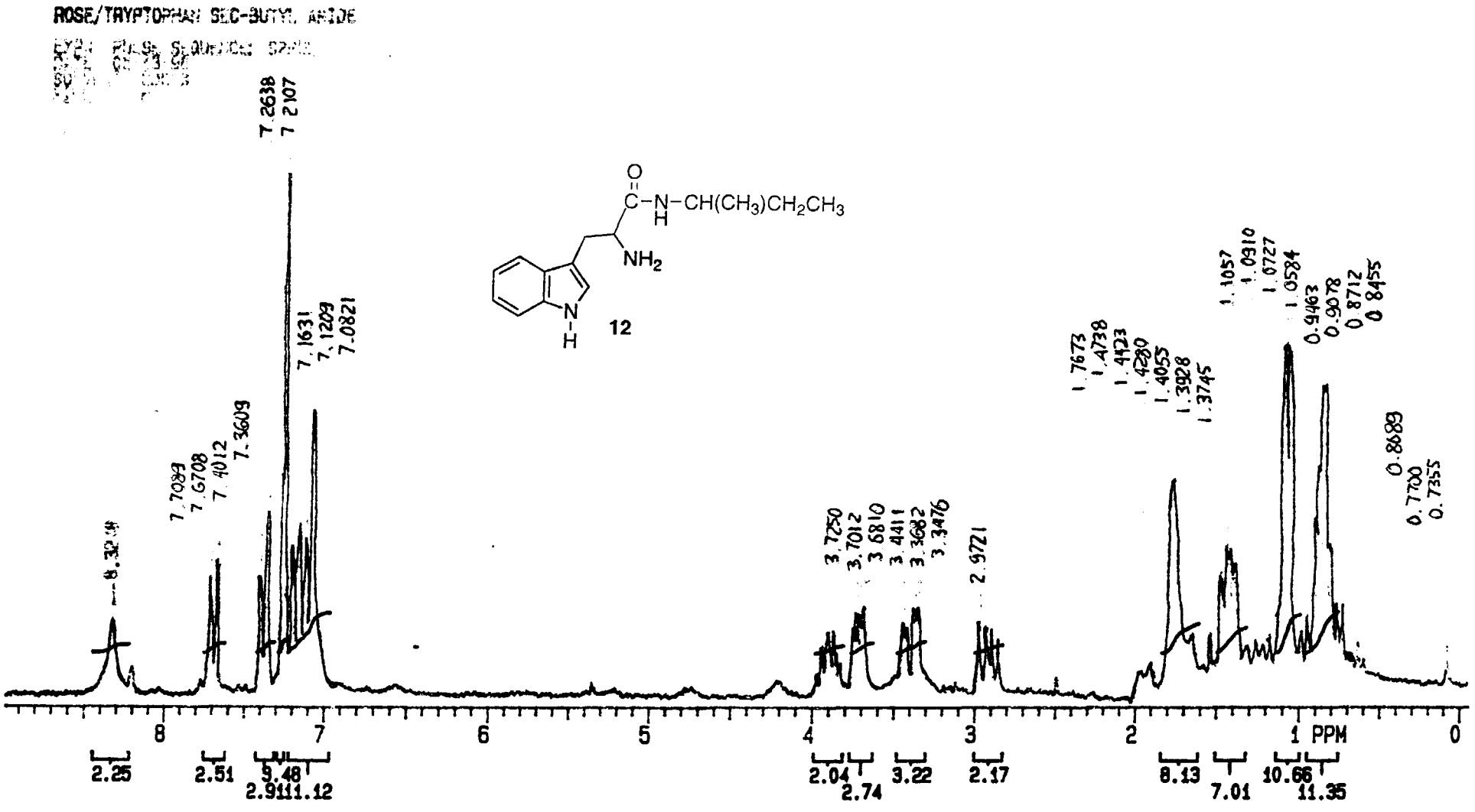
ROSE/TRYPTOPHAN SEC-BUTYL AMIDE
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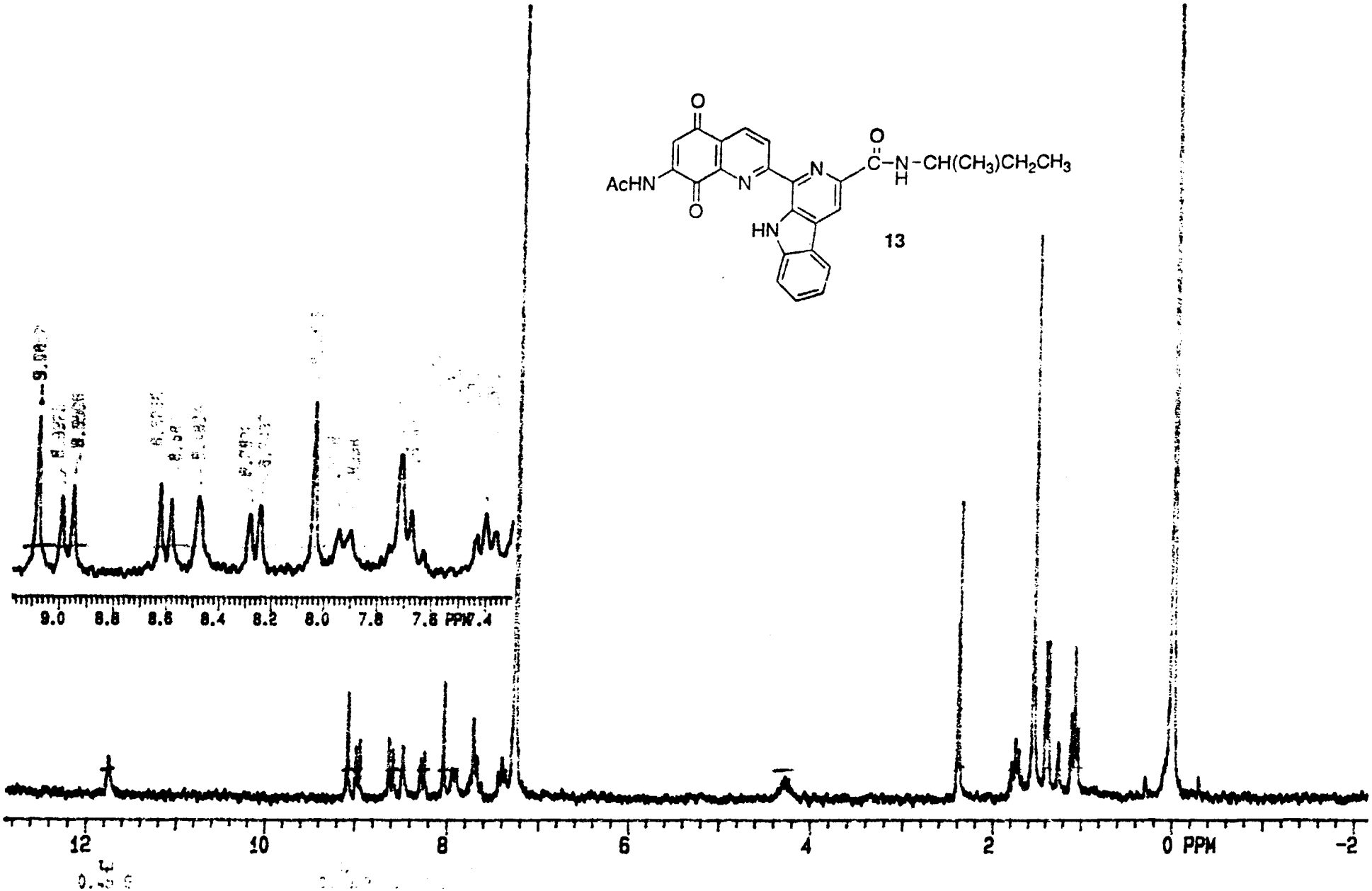
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8.2
8.0
7.8
7.6 PPM
12
10
8
6
4
2
0 PPM
-2

Appendix B - Research Presentations

The author of this thesis presented the work within these pages on two different occasions: once for the Ball State Chemistry Department, and once at the Butler University Undergraduate Research Conference on April 11, 1997. These presentations have served to help the author in the overall understanding of this project, as well as to aid Dr. Mohammad Behforouz in his ongoing studies.

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